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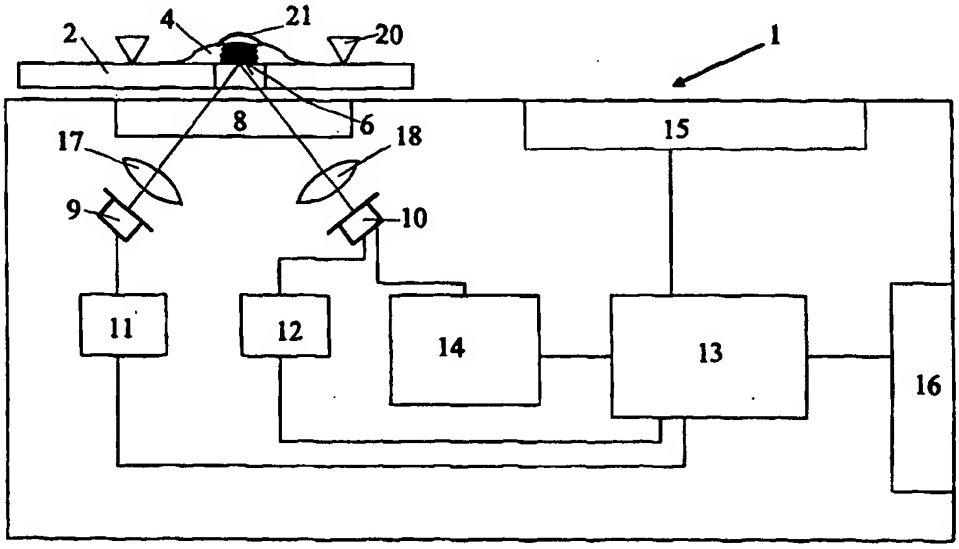

(57) Abstract

The invention relates to a method and apparatus for determining a chemical component from a sample, where the sample is measured on a test strip with the help of a colour reaction. The method concerns finding the measurement time Tm for measuring the reflection of the test strip. The method comprises the following steps: a) Recording the R(t) function, b) Determining the T_0 starting time at the detection of the wetting through of the sample, c) generating the function R(t)+L(t), d) monitoring and storing the ext[R(t)+L(t)] extreme value of the R(t)+L(t) function, and, at the same time, e) generating the function R(t)+L(t)–ext[R(t)+L(t)] from the time of reaching an ext[R(t)+L(t)] extreme value, f) when the R(t)+L(t)–ext[R(t)+L(t)] function reaches a predetermined C(t) value, determining the T_m measuring time.

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METHOD FOR DETERMINING THE GLUCOSE CONTENT OF A BLOOD SAMPLE

Technical Field

The invention relates to a method and apparatus for determining a chemical component from a sample of matter, in particular for determining the glucose content of blood from a blood sample. In the method of the invention, the sample to be measured is positioned on one side of a test strip containing a reagent causing a colour reaction directly or through an intermediate reaction with the chemical component to be measured. The components of the sample penetrate the test strip and start the colour reaction at the other side of the test strip. The content of the component in the sample – in particular the glucose content of the blood sample – is determined by measuring through optical reflection measurement the result of the colour reaction, particularly the developing colour or darkening, and by comparing with earlier calibrating measurements. In the following by colour reaction any chemical or physicochemical reaction is meant, which causes any change in the sample that may be indicated or measured by an optical reflection measurement. That is, the expression "colour reaction" also includes any chemical reaction, where there is no real change of colour - the change of the spectrum of the reflected light - but only the measured intensity is changed, that is some darkening or lightening is detected. Obviously, the above effects may appear mixed.

The invention further relates to an apparatus for determining a chemical component from a sample of matter, in particular for determining the glucose content of a blood sample, particularly for implementing the method according to the invention. The apparatus of the invention comprises a sample holder accommodating the test strip which contains the chemistry for the reaction. The apparatus further comprises a light source illuminating the reaction area of the test strip in the holder – preferably a light emitting diode (LED) –, means for measuring the intensity of the light reflected from the sample– preferably a photo-detector –. The functioning of the apparatus is controlled by programmable controller and analyser means, preferably a microprocessor - for processing the signals of the means for measuring the light

intensity and for determining the chemical component – preferably the glucose content of the blood sample.

Prior art

It is generally widespread and accepted that diabetics are treating themselves in daily life. This is made possible by the use of domestic blood glucose measurement. In the known methods patients with diabetes place a drop of blood on a test strip, which contains the reagent. The reagent will react with the glucose content of the blood, and generates a well-defined colour. The reaction is a multi-stage reaction and is commonly known. The glucose-oxidase enzyme creates hydrogen-peroxide (H_2O_2) from the glucose content of the blood, the oxygen of the air and of the water present in the blood. The amount of the H_2O_2 generated is proportional to the amount of the glucose, and a peroxidase enzyme further activates it. The activated H_2O_2 oxidises the indicator (also commonly known) in the test strip, which will change its colour. This change of colour may be accurately measured.

Earlier test strips have been washed or wiped after the application of the sample, and the colour has been determined by comparison with a colour chart. More recently, the colour has been determined by a small electronic reading device, which calculated automatically the glucose content of the blood sample. The modern test strips are of the so-called no-wipe type, i. e. the blood sample need not be wiped or washed off. With these no-wipe strips the detection of the colour reaction is performed on the opposite side to where the sample has been placed. The test strip is provided with a reagent carrier, usually a textile or foil patch, and the test strip is provide with a hole, through which the opposite side of the reagent carrier may be observed. These test strips are almost exclusively analysed by reading devices, which provide much more objective measurement than the subjective comparison with the colour chart. During the reading, the previous devices have measured the reflection of the reagent carrier on a predetermined wavelength. The colour generated by the colour reaction in the reflection carrier, or more properly on the back side of the carrier, is deduced from the measured reflection value.

The colour reaction on such test strips progresses relatively fast, and both at the start of the reaction and after the completion of the reaction, various effects occur, which affect the results of the colour reaction itself. Therefore, in order to determine precisely the result of the colour reaction, it is important to perform the reflection measurement serving as the basis of the glucose measurement in a well-defined time interval. Only in this manner is it possible to calibrate properly the relation between the colour and the sugar content of the sample.

With the first known devices the measurements were done in the following manner: The patient switched on the device or switched from the stand-by state into the measuring state, after having positioned the sample. This method was not adequate, because the delays until the measurement actually started were varying, due to the switching on, even with by same person. Therefore the need arose to develop such methods, which ensured that the time interval between placing the sample on the test strip and the start of the measurements could be determined uniformly, so that the precision of the measurements could improve. Therefore it is desirable to detect automatically, with the measurement device, the start of the colour reaction and to detect its shape, so that the device could automatically determine a following time interval when the reflection measurement should be performed. The reflection measurement made in this time interval then could serve as a basis for the determination of the glucose content. Alternatively, in the case of continuous or sampled measurement it is sought to determine when the time Tm (time point) occurs, so that a single reflection value measured in this time point Tm could be the basis of the determination of the glucose content. The general object of the present invention is to provide a method for determining this Tm timepoint.

When determining this time point, several factors must be considered, which may present contradictory demands. Of course, it is of primary concern that the Tm time point of the measurement should be determined in a reproducible manner, as well as the R reflection values measured in the Tm time points so determined. The deduced blood glucose values should also be reproducible, i. e. the accuracy of the blood glucose measurements must not be worse than with known methods.

On the other hand, it is desirable to perform the measurement as quickly as possible, which is, firstly convenient for the patient, secondly, the battery in the measurement device may last longer. On the other hand, laboratory measurements have shown that the ideal time point for the measurement is dependent on the glucose content of the sample itself. With certain types of test strips it is advantageous to measure earlier the samples with lower glucose content, than those with a higher glucose content. The reason for this is that with some test strips the colour reaction takes longer with higher glucose content. Conversely, there are test strips where the opposite is true, that is samples with higher glucose content should be measured earlier, because the colour reaction is faster with the higher glucose content and the result is reached earlier. It is advisable to wait longer with low glucose samples until the end of the reaction or close to the end, in order to be able to determine the glucose content precisely. In other words, a good system must be capable of "recognising", even before the final measurement, what the interval should be, and the measurement time Tm can be adjusted accordingly. This is achieved in newer devices by measuring quasi-continuously the reflection curve, and by determining dynamically the final measurement time. This latter process contradicts the requirement for simple operations and calculations. This is an important aspect, because the blood glucose measurement devices should be small and portable (i. e. operating from battery), be simple to operate and last but not least, be cheap. A continuous reflection measurement requires the continuous or frequent switching on/off of the light source, typically a LED, and inevitably have a high power consumption. Therefore, it is sought to substitute the continuous measurement with sampling on a frequency as low as possible. It may also be mentioned that a more complicated method requires a more sophisticated controlling processor, which is more expensive. On the other hand, a more complicated algorithm, in a given processor in a given time, allows the evaluation of fewer measurement points, which in turn will result in a less precise measurement. It is less significant, but may be taken into consideration that the power consumption of the processor is higher with more calculating steps. This latter may play a role if the controlling algorithm of the device is not made by digital processor but by analogue circuits, e. g. due to considerations of reliability. With higher power consumption the device will operate

for a shorter time, so indirectly its reliability will worsen (the probability of malfunction due to the run-down of the batteries will increase).

The document US 4,199,261 (Tidd et al.) discloses an optical reflection meter, which is used to determine the glucose content in urine of diabetics. The device is capable of determining if the sample carrier is dry or wet, by comparing the measured reflection with a predetermined threshold value. The value measured on the dry sample carrier is used for calibrating the device. Following this, the user inserts the sample carrier, which has been wetted with the urine sample, in the device, which is automatically identified by the device. After this the final measurement is made after a predetermined time interval following the recognition of the wet sample.

The documents US 4,935,346 and US 5,049,487 (Phillips et al) disclose a method similar to the previous method, but primarily for determining the sugar content of a blood sample. The device to perform the method is described in the document US 5,059,394. This known method differs from the previous one in that placement of the blood sample - in practice, applying a drop of blood on the sample carrier, in this case a no-wipe test strip - causes the decrease of the reflection, which is detected immediately by the device. Thus the short, but uncertain time interval is excluded, which will necessarily arise in the previously described method of the document US 4,199,261 (Tidd et al.), because of the delay between the wetting of the sample carrier with the urine and placing the sample carrier in the device. In the method described in the document US 4,935,346 the blood sample penetrates the sample carrier, which serves simultaneously as the reagent carrier, and the effective measurement is performed after a predetermined time, following the detection of the decrease in the reflection. This method effectively excludes the subjective elements of the measurement, but its disadvantage is that the measurement time is determined independently of the glucose content. It is a further disadvantage that it needs frequent sampling to determine the exact time of wetting through of the sample, because the reflection curve is falling very steeply around the critical time. If sampling is made at longer intervals, the determination of the To starting time will

be less exact, and from there it follows that the time of the final measurement will also fluctuate in relation to the ideal measurement time determined by the calibration curve. E.g. with higher glucose contents, if the reaction is still in progress in the predetermined measurement time, the uncertainty of the measurement time will be reflected in the measured results.

This latter method has been improved by Phillips et al. according to a method disclosed in the document US 5,179,005. In this known method, based on the theoretical background of the so-called Kubelka-Munk equations, which are well known in the art, the so-called K/S values are calculated, and the blood glucose content is determined on the basis of these K/S values. The final measurement time which serves as the basis of the calculations, is still determined using a predetermined time interval following an initial decrease in the reflection. A disadvantage of this known method is that it is still not able to consider the order of magnitude of the measured glucose content when determining the measurement time. Thus, the measurement is not always made at the ideal time, and further, there must be a trade-off between the accuracy of the measurement and the sampling frequency.

Therefore, it is the object of the invention to provide a method, which allows the determination of the measurement time in a manner avoiding or at least minimising the disadvantages of the known solutions. The objectives of the invention are to determine the measurement time with a simple algorithm, and to determine a measurement time, which is set at or near the ideal time, dependent on the glucose content to be measured. It is a further object to provide a method where the sampling frequency may be kept relatively low, in order to keep the power consumption low. Because the light sources of the measurement devices are normally the largest energy users, this is an important factor. The blood glucose measurements are also negatively affected by temperature variations, hence it is preferable that the method of the invention should deliver results independent of the measurement temperature.

Summary of the invention

According to the invention, the above objectives are realised by a method, whereby the sample is positioned on one side of a test strip, which contains a reagent causing a colour reaction, directly or through an intermediate reaction with the chemical component to be measured. The components of the sample penetrate the test strip and start the colour reaction at the other side of the test strip. The content of the component in the sample – in particular the glucose content of the blood sample – is determined by measuring through optical reflection measurement the result of the colour reaction, particularly the developing colour or darkening, and by comparing with earlier calibrating measurements. According to the invention, the method comprises the steps of:

a) illuminating the sample and measuring at discrete time intervals or substantially continuously the R reflection on the test strip and recording the R(t) function,

b) the detection of the wetting through of the sample, determines the T_0 starting time, T_0 starting time being not earlier than the time of detection of wetting through.

c) generating from the T_0 starting time the function R(t)+L(t) where L(t) is a predetermined function, independent of the measured reflection,

d) monitoring and storing the ext[R(t)+L(t)] extreme value of the R(t)+L(t) function – preferably its min[R(t)+L(t)] minimum value –and, at the same time,

e) generating the function R(t)+L(t)–ext[R(t)+L(t)] from the time of reaching at least one definite (true) ext[R(t)+L(t)] extreme value,

f) when the R(t)+L(t)–ext[R(t)+L(t)] function reaches a predetermined C(t) value, determining the T_m measuring time, and

g) determining from the R reflection value measured at the T_m measuring time the content of the chemical component in the sample, preferably the glucose content of the blood sample.

The method according to the invention is based on the recognition that the sudden reduction in reflection should not be the determining factor, but by utilising the characteristic curve of the reaction being measured, that section must be found where the colour reaction has already come to an end, but where the distorting effect of other phenomena has not yet affected or has only slightly affected the measurement result. We have discovered that at various glucose contents the colour reaction progresses roughly at the same reaction rate. Though this colour reaction does not fully separate in time from the wetting process, however, the manufacturers of test strips basically strive for this. They have already reached a point where, the sections with the maximum reaction rates of the characteristic reactions are separated. The colour reaction involves a characteristic reaction rate, to which a predetermined slope of the reflection curve belongs. Therefore, the appropriate section of the reaction curve must be found which has a predetermined slope, and it can be conveniently found using the algorithm according to the invention. It is apparent that the algorithm is easily programmable and the results can be calculated with a simple, low-performance processor in just a few operational steps. In certain cases, the L(t) value may be presented in tabular form and in this case the processor should perform only subtraction, addition and comparison, instead of division or multiplication. It can be recognised that the T_0 starting time need not be specified exactly, as the aim is merely to ensure that the method should not begin the generation of the $R_{corr}=[R(t)+L(t)]$ value, or at least the search for the extreme value, but only after the lapse of a predetermined period following the commencement of the wetting. It is not necessary to determine the starting time of the wetting very precisely, since the method adjusts the final measurement to a time when the colour reaction has already slowed down. That is, when the reflection curve changes more slowly, therefore, during the search for the minimum value the sampling frequency may be rather low. However, attributable to the rather slow change in reflection, at the same time the exact determination of the final T_m time is less critical than in the already known methods. On the other hand, when the actual values are determined by interpolating the measured values, then the method according to the invention may be applied advantageously for the so-called quick strips, where the reactions

take place quickly. For example, the sampling frequency can be reduced so that the T_m time point is made equal not to the n-th value T_n in which T_n time we would first observe the reaching of the C(t) function, but the exact value of T_m can be a point of time determined by interpolating between the time T_n and T_{n-1}, where T_m is defined by the $F^*(T_m)=C$ value. Here, the function $F^*(t)$ is the linear or higher order approximation of the function $F(t)=R(t)+L(t)-ext[R(t)-L(t)]$ laid through points T_n-1,$F(T_{n-1})$ and T_n,$F(T_n)$. Of course, the measured reflection value R_m is also calculated from the reflection values $R(T_n)$ and $R(T_{n-1})$ by interpolation. The error caused by the interpolation will be very small because in this time interval the variation of R is quite low. A further benefit is that the procedure is less sensitive to the deviations caused by the fluctuation of the reaction rate, because it adjusts the measurement according to the variation in the reaction rate. Therefore, the ageing of the test strips, the measurement temperature, vapour content and other factors affecting the reaction rate will but slightly deteriorate the accuracy of the glucose content measurement.

It is also known that the test strip manufacturers are characterising the different production batches with a so-called code. A code number identifies the characteristics of the test strips of a batch. In order to comply with increasingly exact measurement methods, the manufacturers are using a steadily growing number of the codes. The method according to the invention allows for the adjustment to the fine differences in the characteristics of test strips having many code numbers.

In a favourable embodiment of the method according to the invention L(t) is a linear function with a predetermined slope [$L(t)=At+B$, preferably $L(t)=At$ and $B=0$], while C(t) is a constant function [$C(t)=C$]. However, it is also possible that L(t) is a second-order or a higher order function of time. C(t) can be specified e.g. in a more general polynomial form as well. Preferably, the wetting through of the sample is detected on the basis of a predetermined amount of change in reflection. This requires merely a comparison with a threshold value, therefore, its demand for processing power is rather modest. However, it may be more advantageous if the

wetting through of the sample is detected by a predetermined rate of change in reflection. With this method it is possible to filter out the effects of e.g. the inadvertent moving of the sample and the reductions in reflection not involving a real reaction. Optionally, the wetting of the sample may be detected on the basis of the reflection value reaching a predetermined limit value. This simplifies the programming of the processor, because in the starting phase it requires the storing of a single reflection value at one time.

In an especially preferred embodiment, as blood sample, full blood, blood plasma or serum is applied. In practice it worked well if the illumination was made with an intensity of 0.01 to 1 mW and with a wavelength of 400 to 1500 nm. The intensity and the wavelength of the illumination must be chosen so that the illumination will not affect the progress of the colour reaction through an eventual photochemical reaction.

The invention further relates to an apparatus for determining a chemical component from a sample, in particular for determining the glucose content of a blood sample, particularly for implementing the method according to the invention. The apparatus comprises a sample holder accommodating the test strip applied in the method and entering into chemical reaction with the sample, it further comprises of a light source illuminating the sample placed into the sample holder – preferably a light emitting diode (LED) –, means for measuring the intensity of the light reflected from the sample – preferably a photo-detector –, and further comprises a programmable controller and analyser means, preferably a microprocessor for processing the signals of the means for measuring the light intensity and for determining the chemical component – preferably the glucose content of the blood sample -. According to the invention, the programmable controller and analyser means – preferably microprocessor (13) – is programmed for the execution of the method according the invention.

The invention further relates to a microprocessor readable storage medium with executable instructions of a program for a microprocessor for determining a

chemical component from a sample, in particular for determining the glucose content of blood from a blood sample. The storage medium is storing a program for performing the following steps:

a) measuring at discrete time intervals or substantially continuously the R reflection of the illuminated sample on the test strip and recording the R(t) function,

b) detecting the wetting through of the sample,

c) at the detection of the wetting through of the sample, determining the T_0 starting time, where T_0 starting time is not earlier than the time of detection of wetting through,

d) generating from T_0 starting time the function R(t)+L(t) in which L(t) is a predetermined function, independent of the measured reflection,

e) monitoring and storing the ext[R(t)+L(t)] extreme value of the R(t)+L(t) function – preferably its min[R(t)+L(t)] minimum value –and, at the same time,

f) generating the function R(t)+L(t)–ext[R(t)+L(t)] from the time of reaching at least one definite (true) ext[R(t)+L(t)] extreme value,

g) when the R(t)+L(t)–ext[R(t)+L(t)] function reaches a predetermined C(t) value, determining the T_m measuring time, and

h) determining from the R reflection value measured at the T_m measuring time the content of the chemical component in the sample, preferably the glucose content of the blood sample.

Brief Description of Drawings

The invention is described in more detail with reference to the accompanying drawings showing preferred embodiments of the invention, in particular

Fig. 1 shows the test strip used by the method and apparatus of the invention,

Fig. 2 shows a block diagram of the apparatus according to the invention for performing the method of the invention,

Fig. 3 shows the reflection-time function of samples with different glucose levels, measured by the method,

Fig. 4 shows the standard deviation of several curves measured identically to those of Fig 3,

Fig. 5 shows the Rcorr=(R(t)+L(t)) transformation of the curves of Fig. 3.,

Fig. 6 shows the F(t)=Rcorr-min(Rcorr) transformation of the curves of Fig. 5.,

Fig. 7 shows the relative standard deviation of curves measured with the parameters of Fig. 3.,

Fig. 8 shows the correlation curve, and

Fig. 9 shows the transformed reflection curves of another type test strip, calculated similarly to those of Fig 6.

Best Mode for Carrying out the Invention

Figs 1 and 2 show the test strip 2 used in the method according to the invention, and the apparatus 1 for performing the method. The test strip 2 comprises of a reagent entering into chemical reaction with the component to be determined. In the shown embodiment the apparatus 1 is a blood glucose measuring device, which constitutes a blood glucose measurement system together with the known test strip 2, the latter is a known no-wipe type test strip. Fig 1 shows different views of the test strip 2, from left to right: from the top, bottom and side. As is apparent from Fig. 1, on the top side 3 of the test strip 2 there is a 4 reagent matrix (also called as test pad), which contains the reagent entering into chemical reaction with the sample to be measured. The reagent matrix 4 is made of a textile or foil in a known manner. Below the reagent matrix 4 there is a hole 5 in the test strip 2. The result of the reaction is measured by apparatus 1 through the hole 5 on the lower surface 6 of the reagent matrix 4.

Fig. 2 shows the principal construction of the blood glucose measurement apparatus 1. The test strip 2 is positioned on a external surface of the apparatus, preferably on the top, and fastened temporarily by a known fastening mechanism 20 to the measuring window 8 of the apparatus 1. The test strip 2 is positioned in a manner so

that the hole 5 substantially coincides with the measuring section of the measuring window 8. The apparatus 1 comprises a light source 9, preferably a LED, with well defined properties, and providing substantially monochrome light. The light of the light source 9 is imaged by the beam forming optics 17 onto the lower surface 6 of the reagent matrix 4. The light reflected from the lower surface 6, or a fraction thereof, is measured by the detector 10, in a given case through the imaging optics 18. The values measured by the detector 10 are forwarded via the A/D converter 14 to the microprocessor 13. The glucose content calculated on the basis of the values measured by the detector 10 is displayed on the display 15 and/or transmitted to other data processing units via the interface 16. In practice the 13 microprocessor controls the power supplies 11 and 12, the latter are feeding the light source 9 and the detector 10, respectively. The latter function of the microprocessor 13 is not necessary, but in order to minimise the power consumption it is advantageous to switch on the light source 9 and the detector 10 during the actual measurement only, therefore the power supplies 11 and 12 are switched on by the microprocessor 13.

The blood glucose measuring apparatus of the invention is used in the following manner: Before starting the measurement, the user – typically a diabetic measuring his/her own blood sample – places a test strip 2 on the apparatus 1 and switches on the apparatus. Optionally, the test strip 2 may be placed on the apparatus 1 also after switching on, if the microprocessor of the apparatus also includes an algorithm which is capable of distinguishing between changes in the reflection caused by the placement of the test strip 2 from the changes in the reflection caused by the actual measurement. The latter will be explained more in detail together with the method according to the invention.

After switching on the apparatus 1, the detector 10 measures continuously or at intervals or by sampling in time points the R reflection of the lower surface 6 of the reagent matrix 4. By continuous or substantially continuous measurement it is meant that the sampling frequency of the reflection measurement is as large as possible, i. e. made with at least as large frequency with which the microprocessor 13 or any other controlling and analysing unit is capable of processing the reflection data. If

the algorithm of the invention is realised by analogue circuits, the reflection measurement may be effectively continuous.

Returning to the value of the reflection R measured on the lower surface 6 of the test strip 2, the reflection R will obviously remain constant, until the user places a blood sample – typically a blood drop 21 of capillary blood – on the top surface of the reagent matrix 4, opposite to the lower surface 6. Certain components of the blood drop, especially the blood plasma containing the blood glucose, will penetrate the reagent matrix 4 by wicking action, and will reach the other side, the lower surface 6. As a result, the R reflection measured on the lower surface 6 will change, and this change is detected by the detector 10. The signals of the detector 10 are digitised by the A/D converter 14, and forwarded to the microprocessor 13. The microprocessor 13 is programmed to perform the method according to the invention, and which is described in greater detail below. Therefore, the microprocessor 13 automatically controls the power supply 11 of the light source 9, at the rate of the sampling frequency. At the same time, the 13 microprocessor automatically generates the R(t) function, or more precisely, the R(Ti) points of the R(t) function, and the functions Rcorr(t) and F(t) calculated from the R(t) function. In an other possible embodiment, during the calculation of the Rcorr(t) and F(t) functions, the values of the L(t) and C(t) functions are not calculated for the individual To, Ti, Tmin and Tm and other time points, but the function values are stored in storage unit (not shown) in a tabular form, and the required function values are retrieved from the table.

The R reflection measured by the detector 10 is a result of several different processes, which coincide at least partly, or proceed separately, in time. The first process is the wetting by the fluid, which is primarily a physical process, and the change in the reflection is caused by the change of the refractive index and the surface structure of the reagent matrix 4. This process involves a relatively fast decrease of the reflection R. The next process influencing the reflection is the chemical process actually used to determine the glucose content in the blood. During this reaction the glucose content of the blood will react with a reagent, e. g. the glucose-oxidase enzyme, and further with the water and oxygen, and the resulting substance, the hydrogen peroxide will cause a further colour reaction, the

latter resulting in a characteristic, typically blue colouring on the lower surface 6 of the reagent matrix 4. This blue colouring will appear as further decrease of the reflection R, because the light of the LED, which is radiating in the infrared range, will be reflected less towards the detector 10. This colouring is followed by other processes, which are no longer relevant to the blood glucose measurement, and may actually negatively influence the result of the measurement. Therefore, the reflection R serving as the basis of the glucose content determination should be measured after the end of the second process, but before the start of the disturbing following processes. According to the invention, the Tm measurement time of the reflection measurement used for determining the glucose content is selected in the following manner:

As described above, the detector 10 of the apparatus 1 is measuring continuously in discrete sampling time points Ti the reflection R. Continuously monitoring and analysing the value of the reflection R, it is possible to determine approximately the time point To when the fluid containing the blood glucose wicked through the reagent matrix 4 to the lower surface 6, causes this wetting which produces a sudden drop in the reflection R. This sudden drop may be detected easily, most simply by determining when the reflection R falls below a threshold value. This time point is regarded in the following as the value To=0. Other methods to determine To are also suitable, e. g. when the difference dR=(Rn+1 - Rn) or the ratio dR/dt reaches a predetermined threshold, it may also signal the reaching of the time point To. Alternatively, the wetting may be detected by an electric method, e. g. by a capacitance measurement.

From the time point To, the corrected reflection function Rcorr(t)=R(t)+L(t) is generated, i. e. its actual values in the discrete t time points. The L(t) function is a predetermined function, which may be determined experimentally, or it may be used in the form of an L(t)=At+B linear approximation. The function L(t) also has a reflection dimension, but it is not possible to attribute a direct physical meaning to L(t). Methods to define the function L(t) will be explained more in detail below.

The value of the function Rcorr(t) is also monitored continuously, and the true extreme ext(Rcorr) is sought. Experience shows that the first true extreme of Rcorr will be a minimum, which is reached in the time point Tmin>To. As long as the function Rcorr(t) is decreasing, the extreme value, i. e. the minimum will always be the last Rcorr(t) value. The sought value is the first true extreme, i. e. the first min(Rcorr) value for which it will be first true that

$$\min(Rcorr)=Rcorr(Tmin)<Rcorr(Ti), \text{ if } Ti>Tmin.$$

As soon as this first true extreme min(Rcorr) is found, the function F(t)=(Rcorr(t)-min(Rcorr) is also defined and generated. Obviously, from this time on F(t) will be growing at least on a finite interval, because at the time point Tmin the function Rcorr(t) has a true local minimum. It is understood that the value of the reflection R at the time point Tmin (actually through value Rcorr(Tmin) of the corrected reflection function Rcorr) can be determined exactly, though the exact determination of the time point Tmin is not very important. During the process a relatively low sampling frequency can be applied, because in the proximity of the minimum min(Rcorr) the function Rcorr(t) will be changing relatively slowly, and therefore even a value calculated from interpolation with a second-order polynomial will provide very exact results. The measurements and the practical curves show that the sampling frequency should be adjusted to the processes around the time point Tmin.

The interpolation may be done in the following manner: If it is detected in a certain time point Tk that Rcorr(Tk)>Rcorr(Tk-1), than the value of the function Rcorr(t) is approximated by a second order polynomial Rcorr*(t) laid through the points Tk-2,Rcorr(Tk-2), Tk-1,Rcorr(Tk-1), and Tk,Rcorr(Tk), or through further suitable points, preferably the closest neighbouring points. Higher order approximations are also possible. After calculating the approximated minimum value ext(Rcorr*), further on this value will be regarded as the true minimum value ext(Rcorr) of the function Rcorr(t). In the following the function F(t)=Rcorr(t)-ext(Rcorr) is compared with the function C(t), and the measurement time Tm for measuring the reflection R which will serve as the basis for determining the glucose content is selected as the first time point T, in which the value of the function F(t) reaches or surpasses the value of the function C(t). In other words, Tm is defined as the time

point Tm when it will be first true for t=Tm that F(t)-C(t)≥0, that is when it is first true that F(Tm)-C(Tm)≥0. For even more precise measurements the F(t)-C(t)=0 exact relation is sought, and for this purpose the function F(t) is approximated with the interpolation F*(t), which may be a polynomial of a suitable order. From the relation F*(Tm*)=C(Tm*) the value of Tm* may be calculated exactly, and this interpolated time point Tm* approximates the sought time point Tm with a high accuracy. Of course, the sought reflection value R(Tm) is also calculated with interpolation from the reflection values R(Tj-i),...,R(Tj-1),R(Tj) measured in the time points Tj-i,...,Tj-1,Tj, where the time point Tm is in the time interval Tj-i – Tj. It depends on the construction of the system and may be determined by the person skilled in the art, if the sampling frequency should be higher and the interpolation calculations be kept simpler, or if the accuracy of the measurement should be improved by more sophisticated interpolating but the sampling frequency should be kept low.

The C(t) function may be determined experimentally, but in practice the approximation C(t)=C proved to work well, i. e. C(t) is a constant function. The value of C could be around 0.001-0.05.

Example:

We investigated test strips with samples of test liquid or blood using the above outlined method according to the invention. The test strips used were manufactured by Hypoguard Ltd. of Woodbridge, Suffolk, and sold under the Supreme brand name.

The L(t) function was taken into consideration using the following linear approximation: L(t)=At in which A=2.4 pro mil/sec while the constant C value was 15 pro mil. Figure 3 shows the reflection values measured on different test strips. It can be seen that in samples with high glucose content the reflection falls quicker because the colour reaction also contributes more to the reduction in the reflection. The fact that the two processes are separated in time from each other can be clearly seen in Figure 4. In this latter the standard deviation of the R values of the test strips is presented to the reflection curves measured according to Figure 3. In the measurements sample solutions of 1 to 30 mMol/l glucose concentration were used

(only a few characteristic values of the entire measurement series are shown in the figures). It was presumed that the standard deviation is probably higher in the those parts of the reaction where the reaction rate is higher. Indeed, it can be clearly recognised that the maximum values of the two processes (wetting and colour reaction) are separated from each other in time. It is clear that the change in reflection caused by the wetting process decays very quickly, but the upward going section of the change in reflection attributable to the colour reaction already suppresses this decay. The sought process is the colour reaction, which should be completed before measuring the glucose content. It is evident that while the colour reaction is in progress, the measurement made at that time would cause a high standard deviation. At the same time, after the end of the colour reaction it is advisable to measure as soon as possible, before any further disturbing processes would decrease the correlation between reflection and glucose content. Carrying out the measurement as soon as possible is also desirable from the viewpoint of the user comfort. In Figure 4 it can be seen that the maximum values of the relative standard deviation function cv (R,t) are substantially in the same time interval, and it was determined from other measurements that those sections of the R(t) reflection curve that coincide with the highest reaction rate of the colour reaction have practically the same or similar slope. Therefore, the problem to be solved can be reformulated in the sense that in order to find the maximum of the colour reaction, the point should be found on the reflection curve where the curve has a predetermined slope. This may be found in a very simple way according to the invention, that is, a function with the a predetermined slope value must be added to the reflection curve and a true extreme value of the so defined function must be found. In the used colour reaction the reflection will be reduced, therefore, the sought extreme value will be a minimum. This is illustrated by Figure 5 which presents the values of the corrected reflection function $R_{corr}=R(t)+L(t)$ deduced from the reflection values of Figure 3. The curves obtained after determining the minimum and the transformation of subtracting the minimum value are shown in Figure 6 which shows the actual values of the functions $F(t)=R_{corr}+\min(R_{corr})$. In the lower part of Fig 6. the constant function $C(t)=C$ is shown, marked in the legend by a "nivo" label. As it can be seen, with the test strips applied in this measurement series, it

was possible to achieve, by the appropriate selection of the function L(t), that the measuring system programmed according to the invention will measure samples of high glucose content sooner, those of lower glucose content later. It is seen in Fig. 6 that e.g. with the sample of 2.5 mMol/l concentration the time of T_{min} will fall approximately toward 24 sec, the T_m time to about 38 sec. In the case of a sample of 4.1 mMol/l concentration T_{min} will be near 33 sec while T_m will approximate 46 sec. Comparing this to Figure 3, it can be seen that the measurement of the 4.1 mMol/l sample could be shifted to a time when the developed colour will characterise its glucose content more reliably.

Figure 7 illustrates the standard deviation of test strips measuring samples with high and low glucose concentration. The average standard deviation is a few per cent in samples of low sugar content, this result is not inferior to the standard deviation of other known blood glucose determination methods.

The correlation curve presented in Figure 8 shows the correlation between the T_m times determined on samples with various concentrations and the concentrations calculated from the reflections R at the times T_m. The curve mirrors the results measured and calibrated on a sample series comprising 26 different concentrations, with 20 sample groups with identical concentrations within each concentration range. It is clearly visible that the correlation is very high with short measurement times, e.g. it already reaches 0.96 at 20 sec. The measurements have also shown that the combined error of the optical measurement and the method according to the invention is less than the error introduced into the measurement by the chemical reaction.

Figure 9 shows the F(t) function measured on another type of test strip. It can be seen that in this test strip the samples with lower glucose content reach first the threshold determined by the constant C, that is, the method measures these sooner, while the samples of higher glucose content are measured later, until the completion of the reaction. In this case, at the sample of 2.7 mMol/l concentration, the time of T_{min} falls in the region of about 7 sec, and the T_m time to about 17 sec. In the case of

the sample with 8.55 mMol/l concentration T_{min} falls to about 11 sec and T_m to about 39 sec.

The parameters of the L(t) and C(t) functions of the measurements above were determined as follows:

In order to simplify the measurement and the programming, certain restricting presumptions were introduced:

1. The function L(t) is linear, at t=0 it is 0 (this choice is arbitrary and it does not affect the result). Thus, the determination of the L(t) function was simplified substantially to finding a slope value [L(t)=At+B, but B=0].
2. The function C(t) is constant in time [C(t)=C].

We formulated the problem so that the goal is to determine those "A" and "B" parameters in which the quantity of blood glucose can be determined with the least standard deviation as compared to the reference measurements. For reference measurements various blood glucose measuring instruments using known methods were applied, these were the reference devices. The measurement results obtained with the reference instruments were averaged and this average value was considered as the exact glucose value.

For determining the parameters 1192 reflection-time values were measured, on the Hypoguard Supreme test strips. In one time series we used the most frequent sampling that was permitted by an experimental model of the apparatus according to the invention. In this manner a measured value was generated in every 0.75 sec. Each measured time series contained 100 samplings, so the duration of the individual measurement periods was 100 * 0.75 = 75 sec. The 119,200 data obtained in this way were processed by methods which were known in themselves, using an appropriate spreadsheet program. It must be mentioned that in a given case to optimise a non-linear L(t) function scientifically correctly, a substantially larger number of measurements would be required. The glucose content of the samples used in measurements were in the range 1 mMol/l to 30 mMol/l.

In the first step the value of the C constant was chosen to be 15 pro mil. 1000 pro mil is defined as the reflection value measured on a dry strip without sample. After this, with different A parameters (ranging from 0 to 5 in steps of 1), we calculated the T_m measurement time determined by the formula $F(t)=R(t)+L(t)=\min[R(t)+L(t)]=C$ according to the invention and determined the $R(T_m)$ reflection values at the measurement time T_m, calculating $R(T_m)$ by means of interpolation from the reflection values $R_i(T_i)$ measured in time points T_i around the time Tm.

The $R(T_m)$ reflection values so obtained with the help of the F transformed reflection were converted into mMol/l values using a conversion table, this conversion table was calibrated in a manner similar to the calibration of the conversion tables of the conventional methods. The correlation of the mMol/l values thus obtained were tested against the reference values.

As a result of the first step, we continued to refine the A parameter value from 2 to 3 pro mil, in steps of 0.1. The best correlation was obtained at 2.4 pro mil/sec. After this followed the refining of the C parameter value applying again correlation calculation, with a known recursive approximation algorithm.

Finally, as a result of the calculations, the local maximum of the correlation was obtained at A=2.4 pro mil/sec and C=15 pro mil. The value of the correlation found was 0.96 which is not inferior to the correlation of prior known methods and it can not be further improved, because the chemical processes of the measurement, in particular the standard deviation of the result of the colour reaction already suppresses the standard deviation caused by the optical system and the evaluation algorithm. Another characteristic value is that at A=2 pro mil/sec the correlation is a mere 0.79 while at A=3 pro mil/sec it is already 0.95.

In the above described measurements using the method according to the invention, we measured various test strips using the above described A and C values. We found that the method provided good results even with the test strip for which the

value of the A and C parameters had not been optimised. However, it is obvious that the method according to the invention provides the best results if the parameters of the L(t) and C(t) functions have been calibrated to the test strip to be measured.

Of course, the invention is not confined to the embodiments described above in detail, but other embodiments may also be realised. For example, there is nothing to prevent the method according to the invention, being used in a method using the same principle, but determining glucose, protein or some other component, from a urine sample instead of blood. The method according to the invention can also be used in devices where the wetting of the test strip is not detected automatically or not detected by an optical method, but it is determined by the user or, optionally, it is measured by some other method, e.g. by measuring electric resistance or capacitance. The L(t) and C(t) functions described above are presented as a suggested example, but other functions, like higher order polynomials or various other analytic functions are equally applicable. E. g. C(t) may be chosen in the form C(t)=D-Gt, which will result in the determination of the measurement time Tm not later than Tmin+D/G. This way it is ensured that the measurement will have a definite end under any circumstances.

Claims

1. Method for determining a chemical component from a sample of matter, in particular for determining the glucose content of blood from a blood sample, where the sample to be measured is positioned on one side of a test strip containing a reagent causing a colour reaction, directly or through an intermediate reaction with the chemical component to be measured, where components of the sample penetrate the test strip and start the colour reaction at the other side of the test strip, and where the content of the component in the sample – in particular the glucose content of the blood sample – is determined by measuring through optical reflection measurement the result of the colour reaction , particularly the developing colour or darkening, and by comparing with earlier calibrating measurements,

the method characterised by the following steps of:

a) illuminating the sample and measuring at discrete time intervals or substantially continuously, the R reflection on the test strip and recording the R(t) function,

b) at the detection of the wetting through of the sample, determining the T_0 starting time is, which T_0 starting time is not earlier than the time of detection of wetting through,

c) generating from the T_0 starting time the function R(t)+L(t) in which L(t) is a predetermined function, independent of the measured reflection,

d) monitoring and storing the ext[R(t)+L(t)] extreme value of the R(t)+L(t) function – preferably its min[R(t)+L(t)] minimum value –and, further

e) generating the function R(t)+L(t)–ext[R(t)+L(t)] from the time of reaching at least one definite (true) ext[R(t)+L(t)] extreme value,

f) when the R(t)+L(t)–ext[R(t)+L(t)] function reaches a predetermined C(t) value, determining the T_m measuring time, and

g) determining from the R reflection value measured at the T_m measuring time the content of the chemical component in the sample, preferably the glucose content of the blood sample.

2. Method according to claim 1, characterised by that L(t) is a linear function with a predetermined slope [L(t)=At+B, preferably L(t)=At and B=0].

3. Method according to claim 1, characterised by that L(t) is a second- or higher-order polynomial.

4. Method according to any one of the claims 1 to 3, characterised by that C(t) is a constant function [C(t)=C].

5. Method according to any one of claims 1 to 3, characterised by C(t) being a first- or second-order polynomial.

6. Method according to any one of claims 1 to 5, characterised by detecting the wetting through of the sample on the basis of a predetermined amount of change in reflection or of a predetermined rate of change in reflection.

7. Method according to any one of claims 1 to 5, characterised by detecting the wetting through of the sample on the basis of the reflection value reaching a predetermined limit.

8. Method according to any one of claims 1 to 5, characterised by electrically detecting the wetting through of the sample on the basis of change in resistance and/or capacitance.

9. Method according to any one of claims 1 to 8, characterised by applying as blood sample, full blood, blood plasma or serum.

10. Method according to any one of claims 1 to 5, characterised by applying illumination with an intensity of 0.01 to 1 mW and with a wavelength of 400 to 1500 nm.

11. Apparatus for determining a chemical component from a sample of matter, in particular for determining the glucose content of a blood sample, particularly for implementing the method according to any one of claims 1 to 7, comprising a sample holder accommodating the test strip applied in the method and entering into chemical reaction with the sample, further comprising a light source illuminating the sample placed into the sample holder – preferably a light emitting diode (LED) –, means for measuring the intensity of the light reflected from the sample – preferably a photo-detector –, further comprising a programmable controller and analyser means, preferably a microprocessor for processing the signals of the means for measuring the light intensity and for determining the chemical component – preferably the glucose content of the blood sample -, characterised by that the programmable controller and analyser means – preferably microprocessor (13) – is programmed for the execution of a method comprising the following steps:

a) illuminating the sample and measuring at discrete time intervals or substantially continuously, the R reflection on the test strip and recording the R(t) function,

b) at the detection of the wetting through of the sample, determining the T_0 starting time is, which T_0 starting time is not earlier than the time of detection of wetting through,

c) generating from the T_0 starting time the function R(t)+L(t) in which L(t) is a predetermined function, independent of the measured reflection,

d) monitoring and storing the ext[R(t)+L(t)] extreme value of the R(t)+L(t) function – preferably its min[R(t)+L(t)] minimum value –and,

e) generating the function R(t)+L(t)–ext[R(t)+L(t)] from the time of reaching at least one definite (true) ext[R(t)+L(t)] extreme value,

f) when the R(t)+L(t)–ext[R(t)+L(t)] function reaches a predetermined C(t) value, determining the T_m measuring time, and

g) determining from the R reflection value measured at the T_m measuring time the content of the chemical component in the sample, preferably the glucose content of the blood sample.

12. Apparatus according to claim 11, characterised by comprising storage means for storing the values of the L(t) and/or C(t) functions in tabular form, the storage means being interconnected with the controller and analyser means, preferably a microprocessor (13).

13. A microprocessor readable storage medium with executable instructions of a program for a microprocessor for determining a chemical component from a sample of matter, in particular for determining the glucose content of blood from a blood sample,

characterised by the following steps of the program:

a) measuring at discrete time intervals or substantially continuously the R reflection of the illuminated sample on the test strip and recording the R(t) function,

b) detecting the wetting through of the sample,

c) at the detection of the wetting through of the sample, determining the T_0 starting time is, which T_0 starting time is not earlier than the time of detection of wetting through,

d) generating from the T_0 starting time the function R(t)+L(t) is in which L(t) is a predetermined function, independent of the measured reflection,

e) monitoring and storing the ext[R(t)+L(t)] extreme value of the R(t)+L(t) function – preferably its min[R(t)+L(t)] minimum value –and, at the same time,

f) generating the function R(t)+L(t)–ext[R(t)+L(t)] from the time of reaching at least one definite (true) ext[R(t)+L(t)] extreme value,

g) when the R(t)+L(t)–ext[R(t)+L(t)] function reaches a predetermined C(t) value, determining the T_m measuring time, and

h) determining from the R reflection value measured at the T_m measuring time the content of the chemical component in the sample, preferably the glucose content of the blood sample.

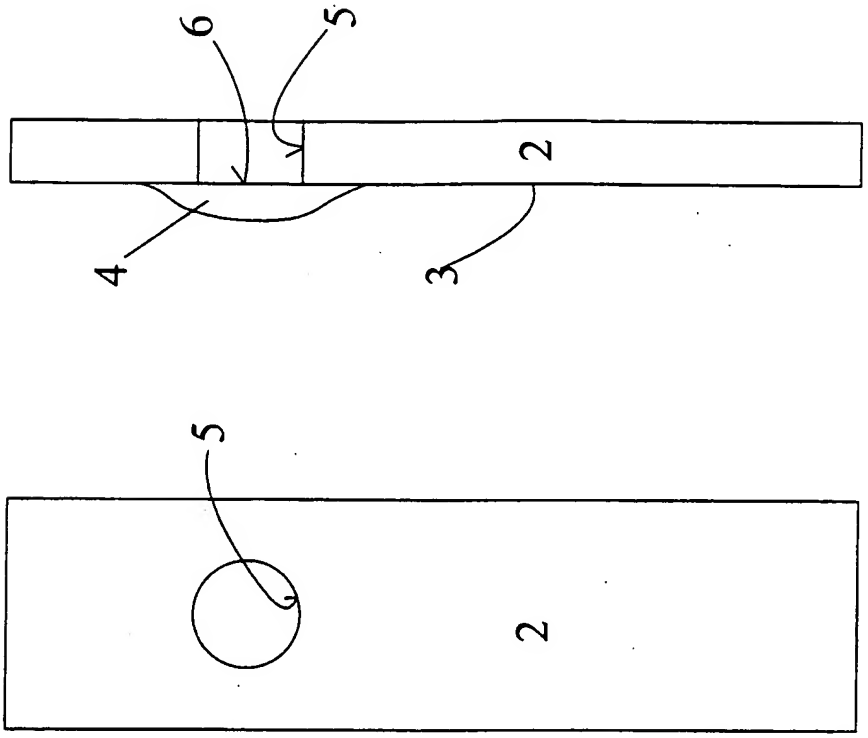


Fig. 1.

Fig. 2.

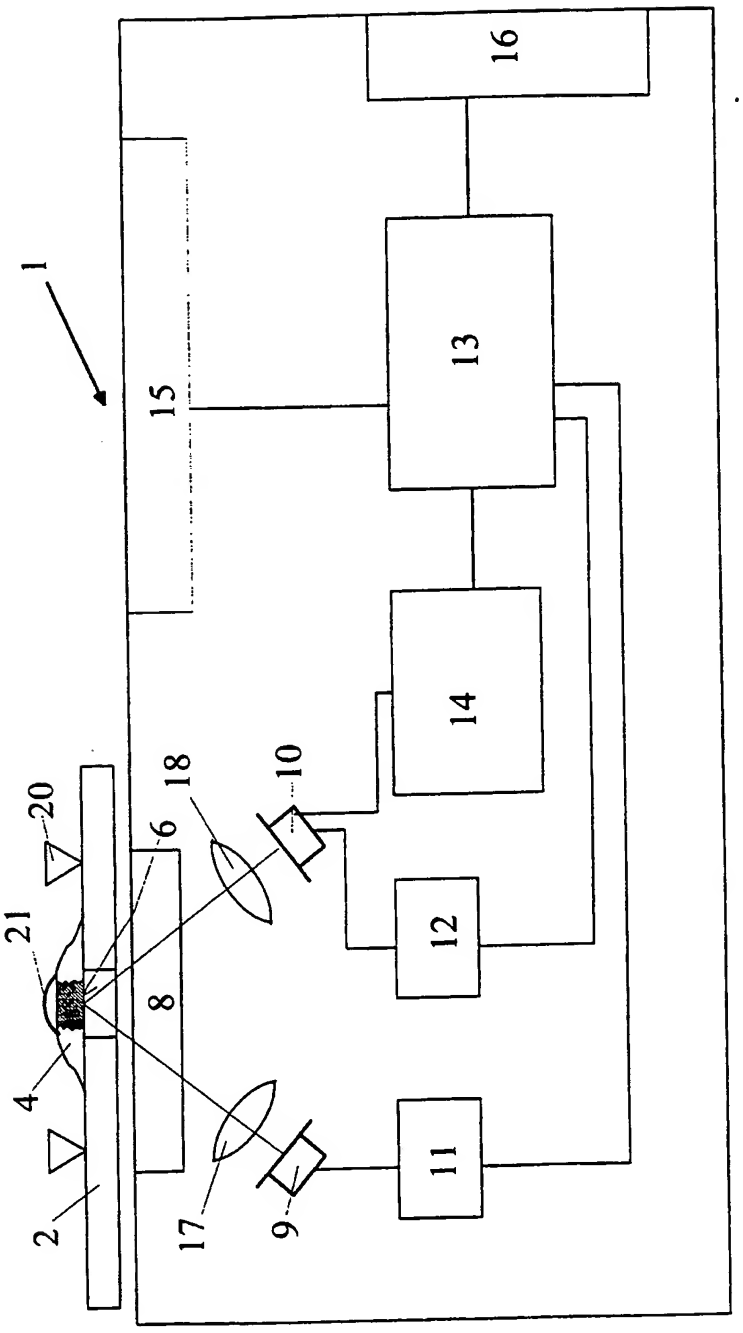
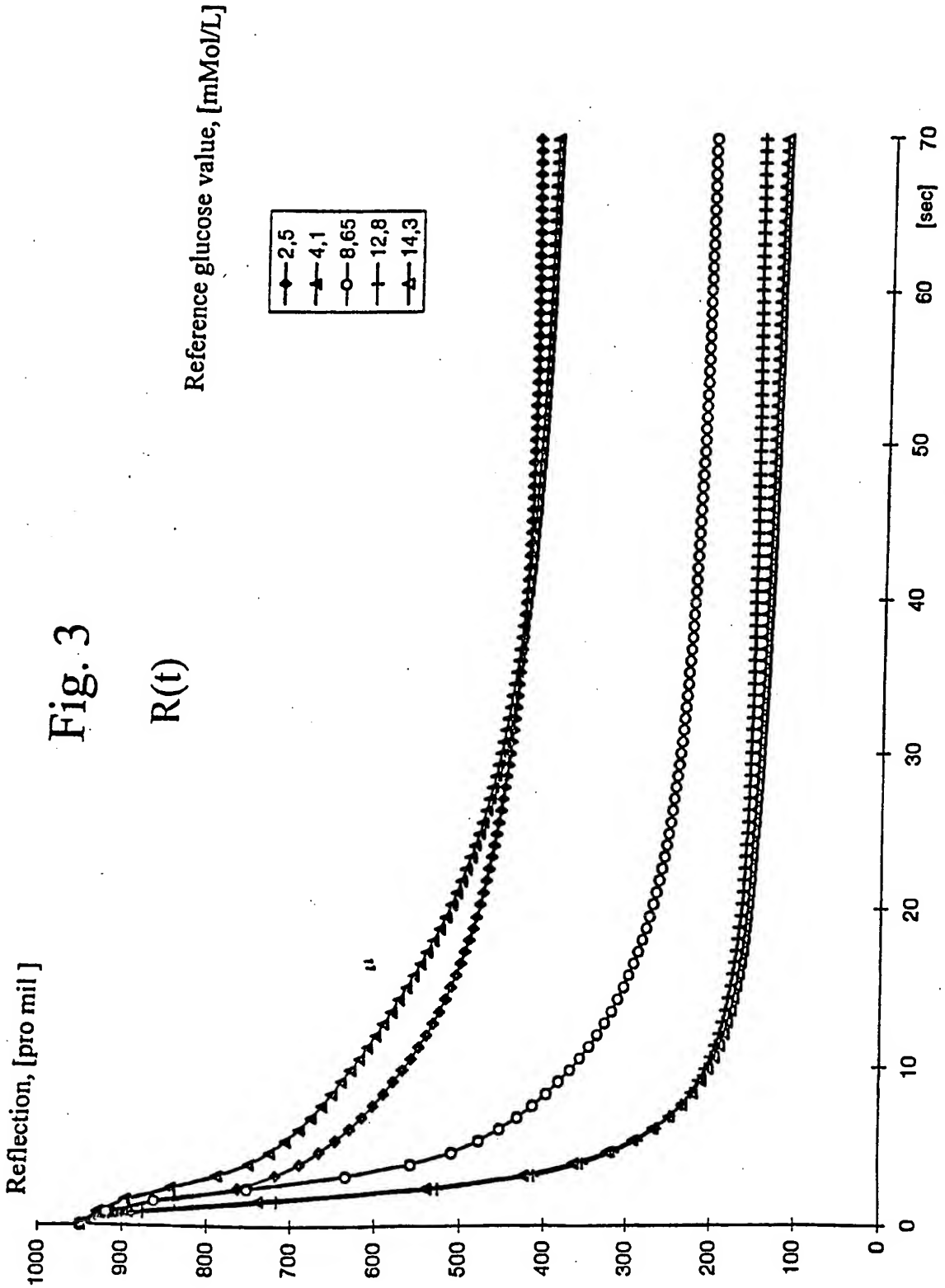


Fig. 3

R(t)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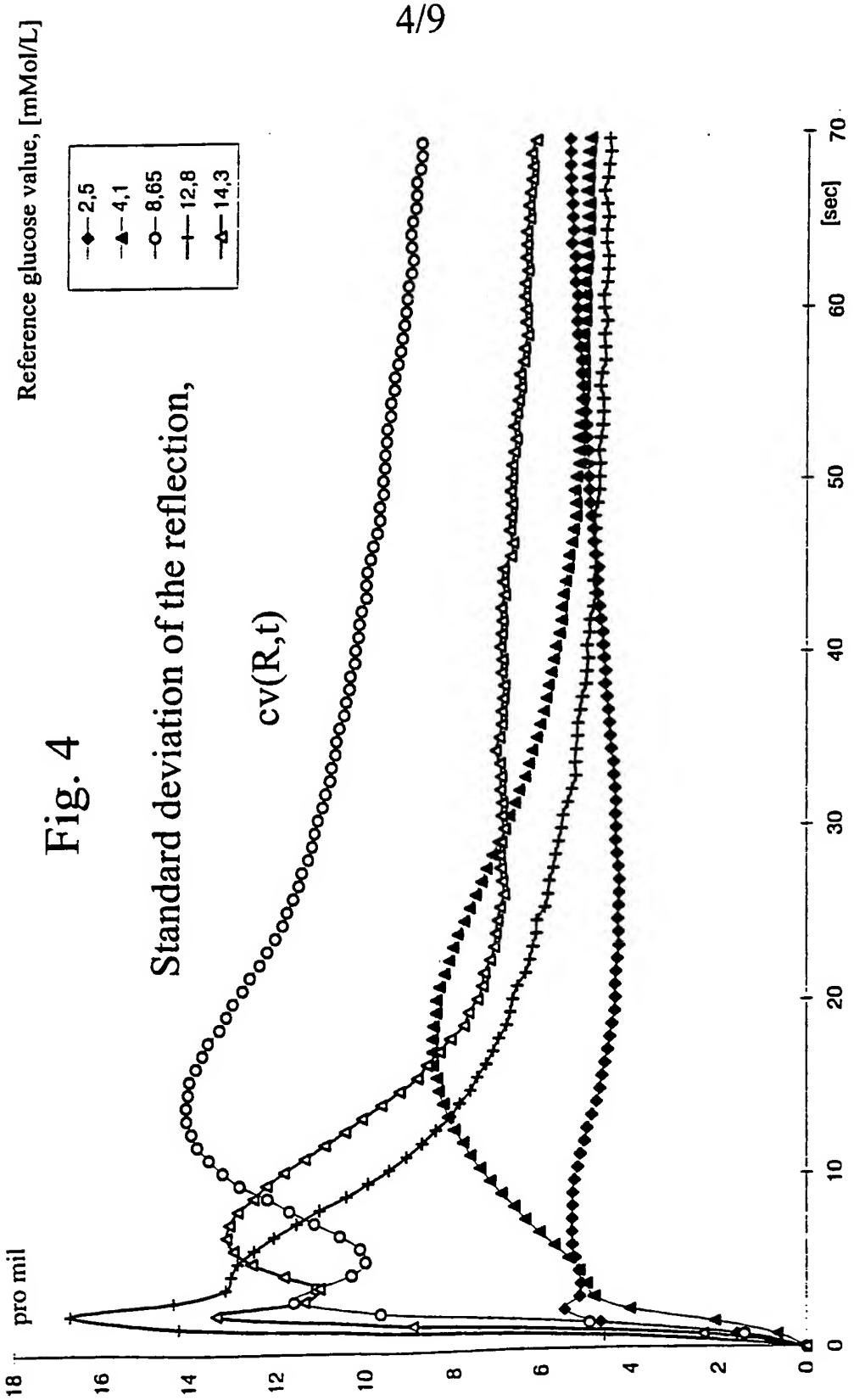
4,1
8,65
12,8
14,3
Reflection, [pro mil]
1000
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
70
[sec]

Fig. 4


Standard deviation of the reflection,
cv(R,t)
Reference glucose value, [mMol/L]
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4,1
8,65
12,8
14,3
pro mil
18
16
14
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
[sec]

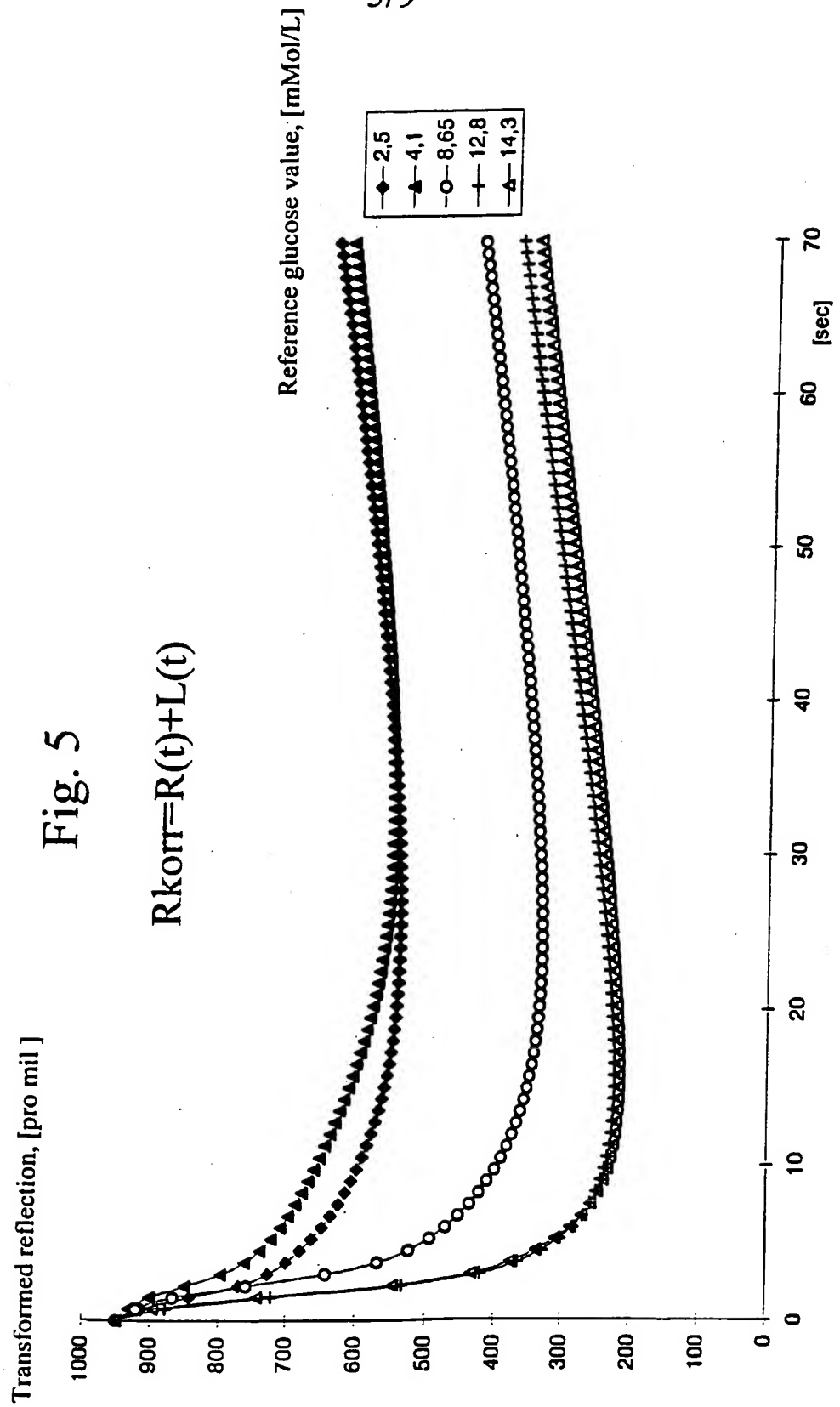


Fig. 5

Rkorr=R(t)+L(t)

Fig. 6

F(t)=Rkorr-min(Rkorr)

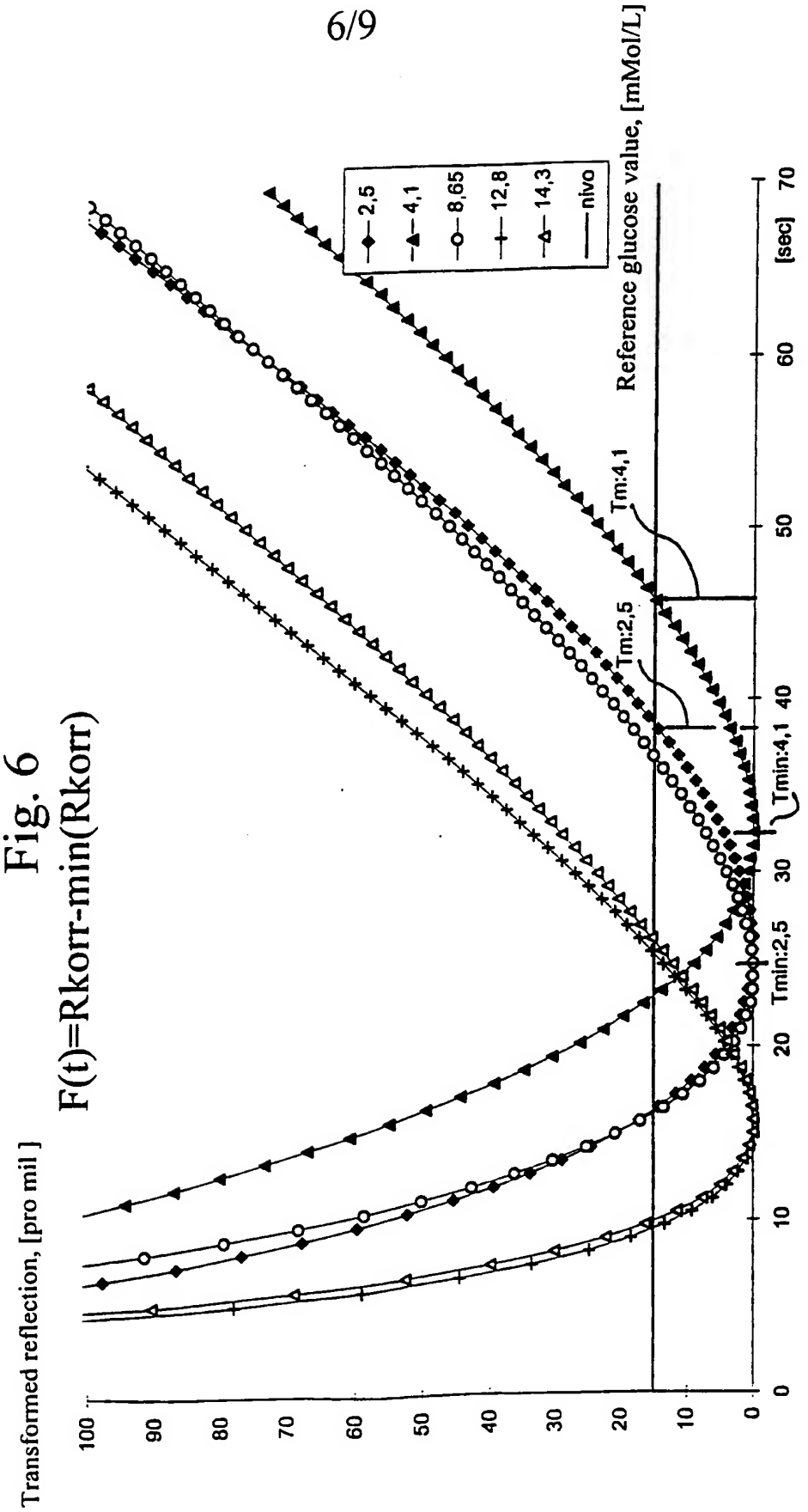


Fig. 7

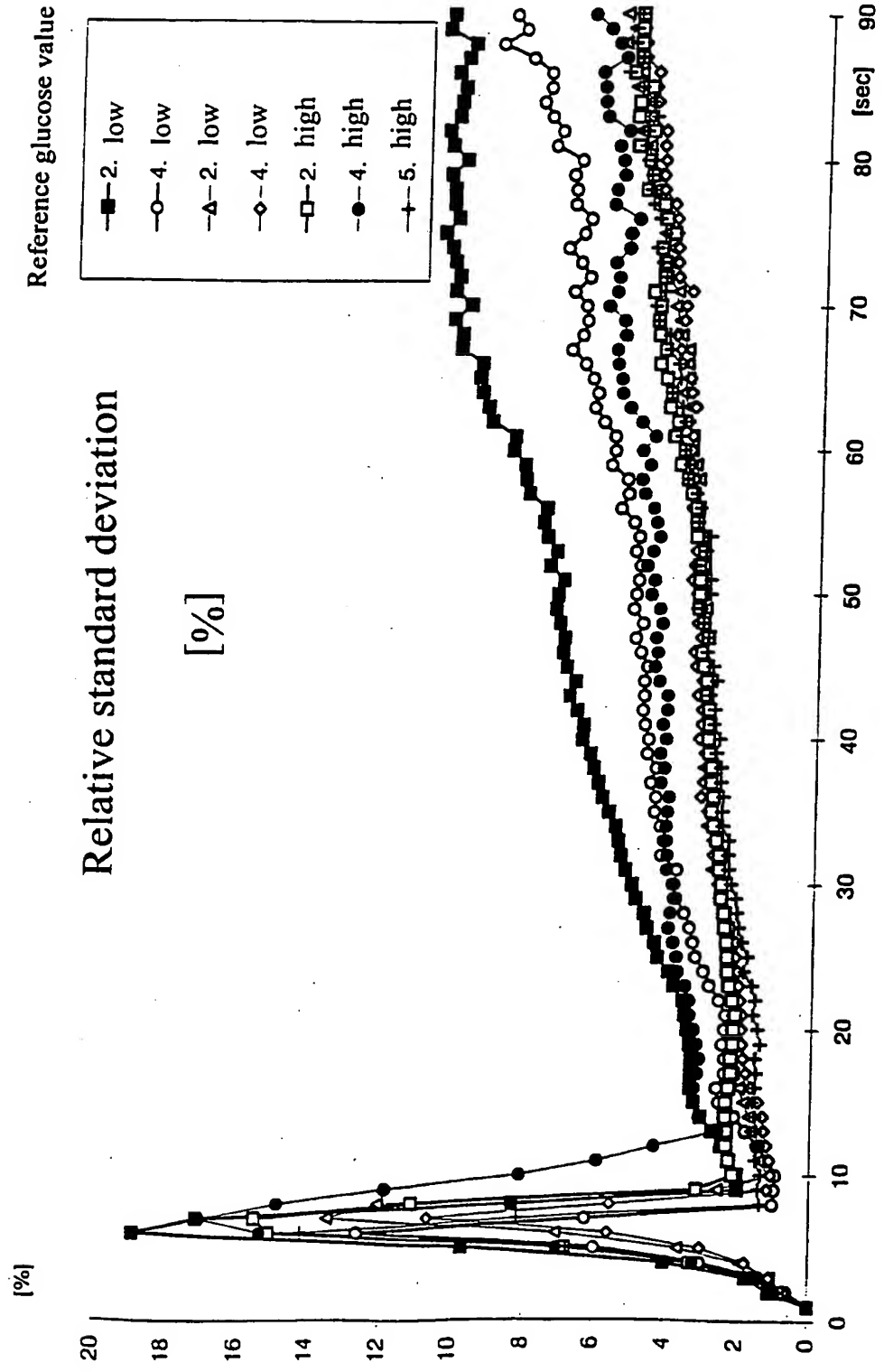

Relative standard deviation
[%]
Reference glucose value
2. low
4. low
2. low
4. low
2. high
4. high
5. high
[%]
20
18
16
14
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
90
[sec]

Fig. 8

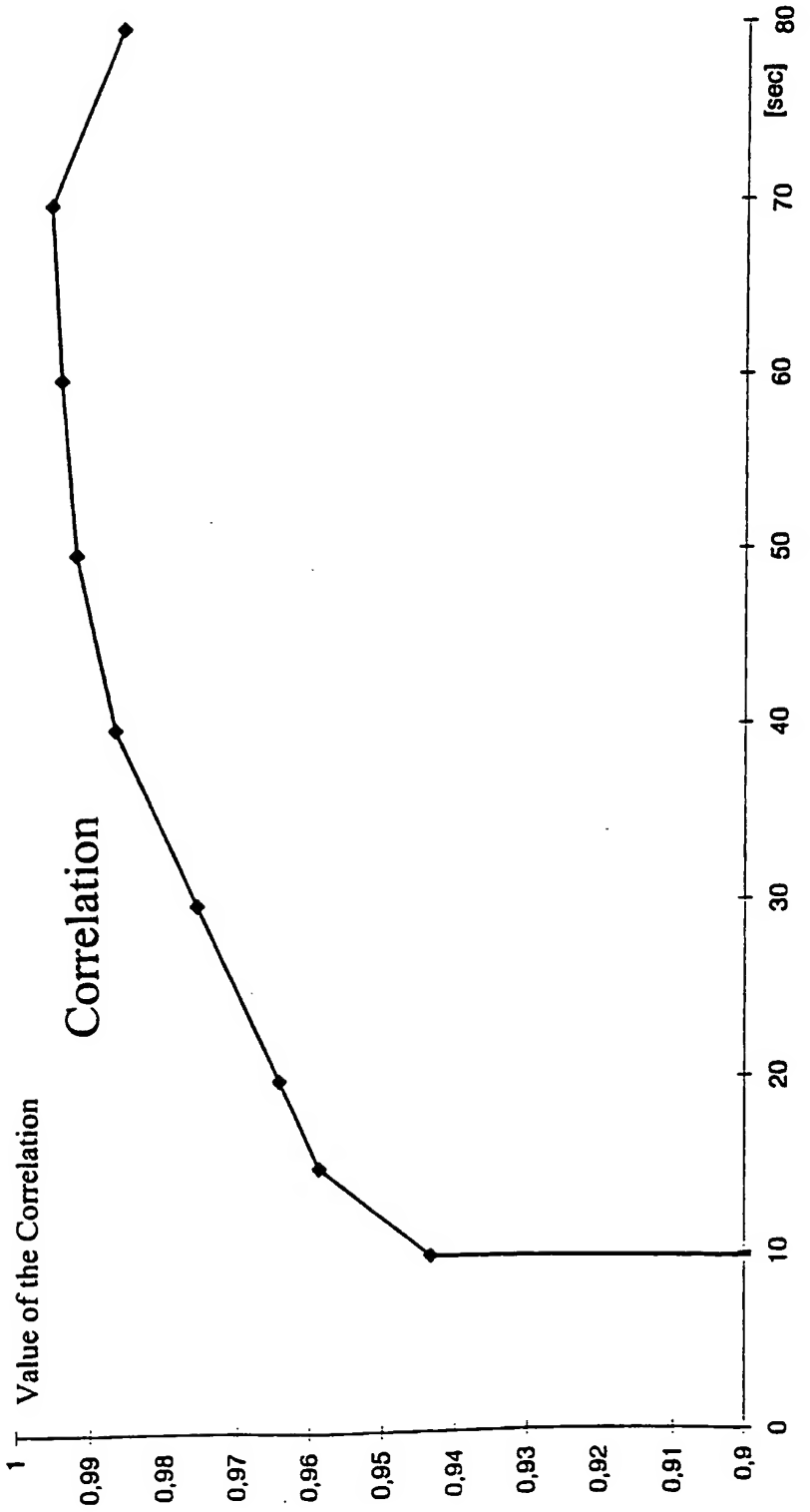
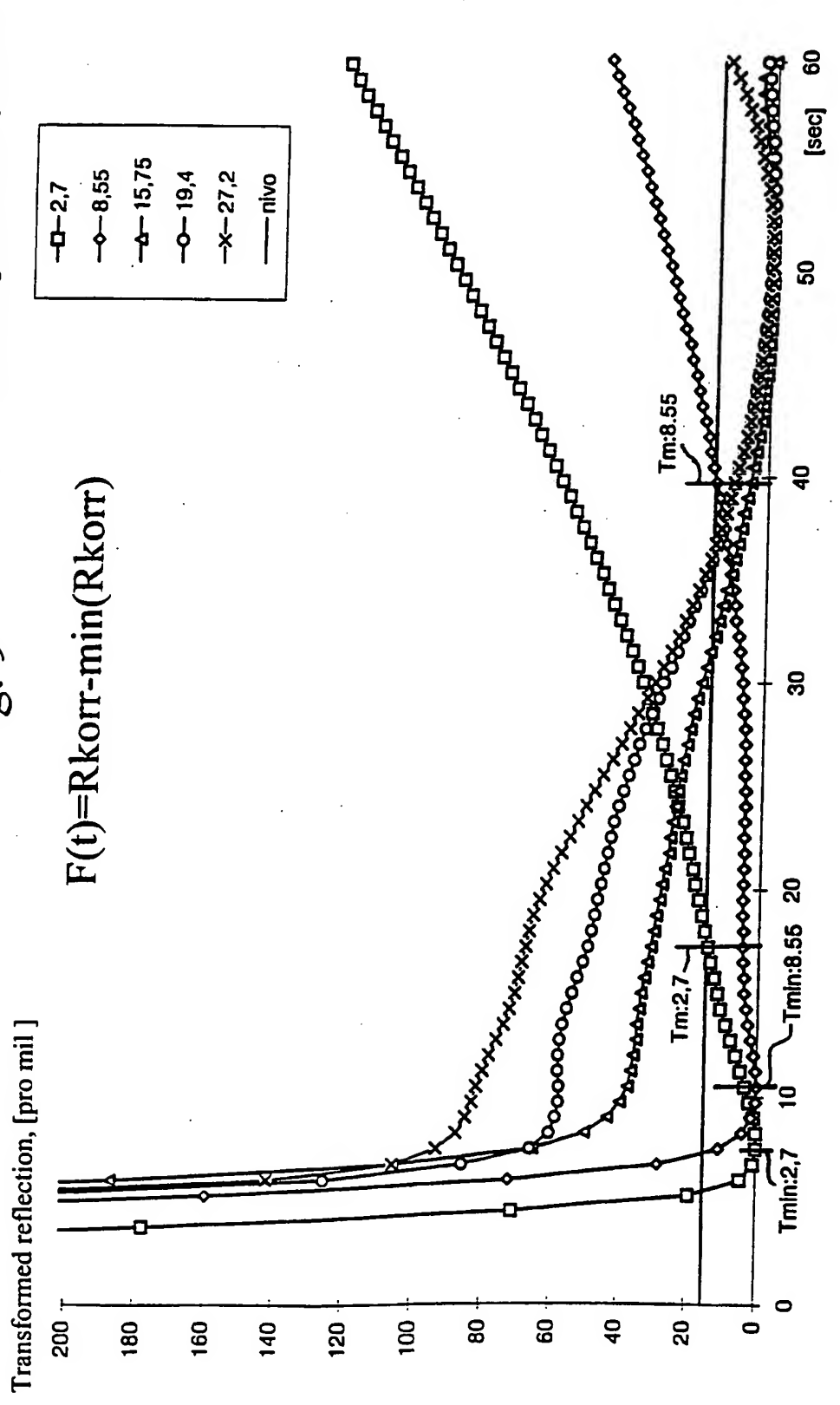

Correlation
Value of the Correlation
1
0,99
0,98
0,97
0,96
0,95
0,94
0,93
0,92
0,91
0,9
0
10
20
30
40
50
60
70
80
[sec]

Fig. 9


F(t)=Rkorr-min(Rkorr)
Reference glucose value, [mMol/L]
2,7
8,55
15,75
19,4
27,2
nivo
Transformed reflection, [pro mil]
200
180
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
[sec]
Tm:8.55
Tm:2,7
Tmin:8.55
Tmin:2,7

INTERNATIONAL SEARCH REPORT

Inter .onal Application No
PCT/HU 98/00091

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/86

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 051 901 A (ENDO) 24 September 1991 see abstract see column 9, line 5 - line 39 see column 10, line 9 - line 18 ---	1,2,4,9, 11,13
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
26 January 1999	03/02/1999

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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